PA 1240105

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

October 26, 2004

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: ***60/533,053***
FILING DATE: ***December 30, 2003***

GB/04/3397

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

P. SWAIN
Certifying Officer

SUBSTITUTE PTO/SB/16 (5-03)

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR §1.53(c).

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Linda Valerie	Thomas	Dorset, United Kingdom
Sébastien	Gouin	DK-Århus, Denmark
John	Faragher	Richfield, WI
Bob	Coyne	Lenexa, KS

Additional inventors are being named on the 0 separately numbered sheets attached hereto

TITLE OF THE INVENTION (500 characters max)

Process

CORRESPONDENCE ADDRESS

Direct all correspondence to:

[X] Customer Number: 26171

OR

[ ] Firm *or* Individual Name					
Address					
Address					
City		State		ZIP	
Country	United States	Telephone		Fax	

ENCLOSED APPLICATION PARTS *(check all that apply)*

[X] Specification *Number of Pages* 43

[] Drawing(s) *Number of Sheets*

[] Application Data Sheet. See 37 CFR 1.76.

[] CD(s), Number

[X] Other (specify) Transmittal Letter in duplicate, 2 pages and 1 page Cover Sheet

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

[] Applicant Claims small entity status. See 37 CFR 1.27.

[X] A check or money order is enclosed to cover the filing fees.

[] The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 06-1050

[] Payment by credit card. Form PTO-2038 is attached.

FILING FEE AMOUNT ($) $160.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[X] No.

[] Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

Signature _____ Date December 30, 2003

Typed Name Harold H. Fox, Reg. No. 41,498

Telephone No. (202) 783-5070

Docket No. 16137-006P01

40195893.doc

Attorney's Docket No.: 16137-006P01 / P019170USO

PROVISIONAL APPLICATION FOR PATENT

under

37 CFR §1.53(c)

TITLE: PROCESS

APPLICANT: LINDA VALERIE THOMAS, SÉBASTIEN GOUIN, JOHN FARAGHER AND BOB COYNE

PROCESS

The present invention relates to a process for introducing an antimicrobial material into a foodstuff. The present invention further relates to an antimicrobial material.

Background

Bacteriocins are antimicrobial proteins or peptides that can be produced by certain bacteria, which can kill or inhibit the growth of closely related bacteria. The bacteriocins produced by lactic acid bacteria are of particular importance since they have great potential for the preservation of food and for the control of foodborne pathogens. (Wessels et al. 1998.)

The most well known bacteriocin is nisin, which is the only bacteriocin currently authorised as a food additive. Nisin is produced by fermentation of the dairy starter culture bacterium *Lactococcus lactis* subsp. *lactis*, and is sold as the commercial extract Nisaplin® Natural Antimicrobial (Danisco). Nisin has an unusually broad antimicrobial spectrum for a bacteriocin, being active against most Gram-positive bacteria (e.g. species of *Bacillus*, *Clostridium*, *Listeria*, lactic acid bacteria). It is not normally effective against Gram-negative bacteria, yeasts or moulds. Nisin is allowed as a food preservative worldwide but its levels of use and approved food applications are strictly regulated, varying from country to country.

Other bacteriocins have since been discovered with potential as food preservatives, e.g. pediocin, lacticin, sakacin, lactococcin, enterococin, plantaricin, leucocin. These are also active, although usually with a more narrow spectrum, against Gram-positive bacteria. Their food use is at present restricted to production of the bacteriocin *in situ*, i.e. by growth of the producer organism within the food.

Food safety and prevention of food spoilage is an ever present concern worldwide, particularly with the increasing trend for convenience foods such as ready to eat meals, soups, sauces or snacks. Spoilage of food is a major economic problem for the food manufacturer. Food manufacturers need to protect the health and safety of the public by delivering products that are safe to eat. Such food must have a guaranteed shelf life, either at chilled or ambient temperature storage. Consumers prefer good tasting food of

high quality - this is difficult to achieve with chemical preservatives, harsh heating regimes and other processing measures. Food safety and protection is best achieved with a multiple preservation system using a combined approach of milder processing and natural preservatives. Foodborne micro-organisms are also less able to adapt and grow in food preserved with different preservative measures.

There is much concern about food safety and the growth of food pathogens such as *Listeria monocytogenes*. This particular pathogen can grow at low temperatures, which are often used as an additional preservative measure. Foodborne pathogens can sometimes adapt to different preservatives and storage conditions, thus a combination of preservative measures can be more successful than individual measures.

Cooked meat joints are new generation, convenience products now on offer to consumers. The preparation of these meat joints usually involves injection or tumbling of the raw meat in polyphosphate brine to increase the meat's tenderness, moistness and volume. The meat is then cooked before distribution to retail outlets and its subsequent consumer purchase and consumption.

The majority of processes for these meats now involve the 'cook-in' system in which the meat is cooked in plastic bags or film. Whole joints may be de-boned, pumped with polyphosphate brine and tumbled or massaged for a short period. This distributes the brine evenly and also achieves a layer of exudate on the surface that helps the plastic packaging to adhere closely to the meat surface. Large joints are usually gas or vacuum-packaged into plastic bags. These cooked meat products are often considered to be of good quality and healthy, since they may be low in fat with minimal salt content. They may not necessarily be re-heated by the purchaser prior to consumption.

These minimally processed products rely on refrigeration to ensure stability and safety of the cooked meat during shelf life, which may be as long as 90 days (Varnam and Sutherland, 1995). Spoilage of the cooked meats, if post-processing contamination is not a factor, would be due to the Gram-positive heat-resistant bacteria *Bacillus* and *Clostridium*, particularly if the meat is exposed to temperatures above 7 °C. Spoilage due to these organisms can be rapid if the meat is exposed to temperatures as high as 15 °C or above. If the meat has not been sufficiently cooked, *Enterococcus* or heat-resistant *Lactobacillus* species may survive, many of which can grow at refrigeration

temperatures. If the product has been handled after cooking then re-packaged and vacuum-packed, spoilage is often associated with *Lactobacillus*, *Leuconostoc* or *Carnobacterium*. *Brochothrix thermosphacta*, another Gram-positive bacterium, can also cause problems. Gram-negative bacteria will only be a problem in unpackaged cooked meats, or those that are packed in air-permeable film. Moulds may develop on cooked meat joints that have been exposed to air and whose surfaces have dried out. There is also concern over post-processing contamination and growth of *Listeria monocytogenes*, a foodborne pathogen that can grow at refrigeration temperatures. It would be a benefit to both the public in terms of safety and manufacturers in terms of economics and reputation, if an effective preservative could be somehow applied to the surface layer of the cooked meat.

Raw, whole muscle meat is also being increasingly sold as a chilled convenience meat product that is ready prepared and tenderised for the consumer to cook. The meat is usually covered with a marinade then vacuum-packed in a clear pouch. The marinade may be applied and simply left to soak into the meat surface, or the meat may be tumbled in the marinade to increase its tenderising effect and penetration. This vacuum-packed, marinated fresh meat can be kept for up to 28 days at refrigeration temperatures before purchase by the consumer and subsequent cooking at home. These meat products are considered value-added fresh meats and cover a wide range of raw meats (pork, chicken, beef, ground beef, steaks, diced meats, joints, etc.). The combination of the acidic nature of the marinade and the lack of oxygen in the vacuum-packed pouches means that Gram-positive lactic acid bacteria are associated with spoilage of these products (Susiluoto et al. 2003).

Nisin is a natural preservative that has been used safely in food for nearly 50 years. It is effective against Gram-positive bacteria including lactic acid bacteria, *Brochothrix thermosphacta*, *Listeria monocytogenes*, *Bacillus* and *Clostridium*. As the spoilage associated with both the meat products described above, is usually caused by Gram-positive bacteria, nisin could be considered as part of a preservative system to guarantee or extend shelf life. However the environment of both meat products is not favourable to nisin stability or activity. Brine and polyphosphate solutions used to inject raw meat are usually at alkaline pH. Nisin stability is optimum at pH 3 (Davies et al. 1998). The cooking process, particularly at high or neutral pH conditions, would lead to significant nisin degradation. In raw meat, nisin is vulnerable to degradation by proteases. A more

specific concern is the inactivation of nisin in raw meat by the formation of an adduct with glutathione in an enzyme-mediated reaction (Rose et al. 1999, 2002, 2003).

Numerous prior art teachings have discussed potential uses of nisin in foodstuffs. Examples are:

- Caserio et al. (1979) describes research on the use of nisin in cooked, cured meat products. Mortadella, wurstel sausage, prosciutto. The target organisms: *Staphylococcus*, sulphate-reducing clostridia. Prosciutto had nisin injected with brine after dissolution in dilute lactic acid.
- Gola (1962) incorporated nisin into the gelatine for canning of large hams. In the first experiment, brines for injection were acidified to facilitate nisin solubility.
- Taylor & Somers (1985) evaluate the antibotulinal effectiveness of nisin in bacon. Nisin was included in brine formulation injected into pork belly.
- Usborne et al. (1986) discusses sensory evaluation of nisin-treated bacon. Nisin was added to brine pumping solution before injection into the bacon.
- US 2003/0108648 relates to compositions having bacteriostatic and bactericidal activity against bacterial spores and vegetative cells and process for treating foods therewith.
- US 6207210 relates to broad-range antibacterial composition and process of applying to food surfaces
- EP0770336 describes injection of meat trimmings/brine solution in which a starter culture has produced a bacteriocin.
- Article found at http://www.nal.usda.gov/fsrio/ppd/ars010f.htm on work at Meat Research Unit, MARC mentions a presentation on 'antibacterial properties of injectable beef marinades'.
- WO2003/11058 relates to food preservation formulation comprising compound(s) derived from natural sources. Natural compounds are formulated and application to a food and irradiation at < 3kGy results in decrease of microflora compared to non-irradiated controls. Nisin is a preferred compound.
- US 2003/0108648 teaches nisin as part of a combination for marinades

The above extensive prior art does not address or solve the problems of protection of antimicrobial materials such as nisin from environments, such as those in meat products, which are not favourable to the stability or activity of the antimicrobial material

The present invention alleviates the problems of the prior art.

In one aspect the present invention provides a process for introducing an antimicrobial material into a foodstuff comprising (i) providing the antimicrobial material in an encapsulated form comprising a core of antimicrobial material and shell of encapsulating material, (ii) introducing encapsulated antimicrobial material into the foodstuff by (a) injecting the encapsulated antimicrobial material into the foodstuff or (b) tumbling the encapsulated antimicrobial material with the foodstuff.

In one aspect the present invention provides a foodstuff prepared by a process for introducing an antimicrobial material into a foodstuff comprising (i) providing the antimicrobial material in an encapsulated form comprising a core of antimicrobial material and shell of encapsulating material, (ii) introducing encapsulated antimicrobial material into the foodstuff by (a) injecting the encapsulated antimicrobial material into the foodstuff or (b) tumbling the encapsulated antimicrobial material with the foodstuff.

In one aspect the present invention provides a foodstuff obtainable by a process for introducing an antimicrobial material into a foodstuff comprising (i) providing the antimicrobial material in an encapsulated form comprising a core of antimicrobial material and shell of encapsulating material, (ii) introducing encapsulated antimicrobial material into the foodstuff by (a) injecting the encapsulated antimicrobial material into the foodstuff or (b) tumbling the encapsulated antimicrobial material with the foodstuff.

In one aspect the present invention provides an antimicrobial material in an encapsulated form, comprising a core of antimicrobial material and shell of encapsulating material, wherein the shell of encapsulating material is impermeable to the antimicrobial material.

The term "encapsulated" is well known in the art. Encapsulation can be defined as the technology of packaging a substrate (solids, liquids, gases) within another material. In the encapsulate the material which has been entrapped is termed the core material or the internal phase while the encapsulating material is referred to as the coating, the shell material or the carrier. Such encapsulated materials are also commonly referred to as core/shell materials.

Aspects of the invention are defined in the appended claims.

We have found that by providing the present antimicrobial materials in an encapsulated form the materials may be protected from environments, such as those in meat products, which are not favourable to the stability or activity of the antimicrobial material. Moreover, by injecting the encapsulated antimicrobial material into the foodstuff or tumbling the encapsulated antimicrobial material with the foodstuff, the encapsulated antimicrobial material may be effectively introduced into the foodstuff. We have found the injection particularly advantageous and surprising. Prior art processes have directly injected non-encapsulated antimicrobial materials such as nisin into food products. We have found that a "shell" may be provided on the antimicrobial material which is capable of withstanding the demanding physical forces exerted on the encapsulated antimicrobial material during injection. In particular injection exerts high pressures and yield stress on the material to be injected. We have also found that a "shell" may be provided on the antimicrobial material which is capable of protecting the antimicrobial material from adverse conditions and/or allows sustained/controlled release.

The present invention provides a process for delivering an antimicrobial material and an anti-microbial material per se which is resistant to unwanted degradation and which may be released to provide a long term antimicrobial effect.

For ease of reference, these and further aspects of the present invention are now discussed under appropriate section headings. However, the teachings under each section are not necessarily limited to each particular section.

PREFERRED ASPECTS

ANTIMICROBIAL MATERIAL

In one preferred aspect the antimicrobial material is an antibacterial material.

In one preferred aspect the antimicrobial material is a bacteriocin.

The antimicrobial material, such as a bacteriocin, may typically be selected from materials (bacteriocins) that can be used as preservatives in food

Preferably the antimicrobial material is selected from lanthionine containing bacteriocins, *Lactococcus*-derived bacteriocins, *Streptococcus*-derived bacteriocins, *Pediococcus*-derived bacteriocins, *Lactobacillus*-derived bacteriocins, *Carnobacterium*-derived bacteriocins, *Leuconostoc*-derived bacteriocins, *Enterococcus*-derived bacteriocins and mixtures thereof.

Preferably the antimicrobial material is at least nisin.

Preferably the antimicrobial material consists of nisin.

Nisin is a lanthionine-containing bacteriocin (US 5691301) derived from *Lactococcus lactis* subsp. *lactis* (formerly known as *Streptococcus-lactis*) (US 5573801). In a preferred aspect of the present invention the bacteriocin used in the present invention is at least nisin.

As discussed in US 5573801 nisin is a polypeptide bacteriocin produced by the lactic acid bacteria, *Lactococcus lactis* subsp. *lactis* (formerly known as *Streptococcus lactis* Group N).

Nisin is reportedly a collective name representing several closely related substances which have been designated nisin compounds A, B, C, D and E (De Vuyst, L. and Vandamme, E. J. 1994. Nisin, a lantibiotic produced by *Lactococcus lactis* subsp. *lactis*: properties, biosynthesis, fermentation and applications. In: Bacteriocins of lactic acid bacteria. Microbiology, Genetics and Applications. Eds.: De Vuyst and Vandamme. Blackie Academic and Professional, London). . The structure and properties of nisin are also discussed in the article by E. Lipinska, entitled "Nisin and Its Applications", The 25th Proceedings of the Easter School in Agriculture Science at the University of Nottingham, 1976, pp. 103-130 (1977), which article is hereby incorporated by reference. In 1969 the FAO/WHO Joint Expert Committee on Food Additives set specifications for the purity and identity of nisin (FAO/WHO Joint Expert Committee on Food Additives. 1969. Specifications for identity and purity of some antibiotics. 12th Report. WHO Technical Report Series No. 430). This committee recognised nisin as a safe and legal preservative based on extensive toxicological testing. Nisin has the food additive number E234 and is classed as GRAS (Generally Recognised As Safe) (Food and Drug Administration. 1988. Nisin preparation: Affirmation of GRAS status as a

direct human ingredient. Federal Regulations 53: 11247). The international activity unit (IU hereinafter) was defined as 0.001 mg of an international nisin reference preparation. Nisaplin® Natural Antimicrobial is the brand name for a nisin concentrate containing 1 million IU per g, which is commercially available from Danisco.

Nisin is an acknowledged and accepted food preservative with a long history of safe, effective food use. There have been several reviews of nisin, e.g. Hurst 1981; 1983; Delves-Broughton, 1990; De Vuyst and Vandamme, 1994; Thomas *et al.* 2000; Thomas & Delves-Broughton, 2001). Nisin was discovered over 50 years ago and the first commercial preparation, made in 1953, was Nisaplin®. Nisin has several characteristics that make it particularly suitable as a food preservative. It has undergone extensive toxicological testing to demonstrate its safety. It is heat-stable, acid-stable and effective against a broad spectrum of Gram-positive bacteria. It is not normally effective against Gram-negative bacteria, yeasts or moulds but activity against Gram-negative bacteria and yeasts has been reported in the presence of chelating agents (PCT/US 8902625. WO 89/12399). Nisin is an effective preservative in pasteurised and heat-treated foods (e.g. processed cheese, cheese, pasteurised milks, dairy desserts, cream, mascarpone and other dairy products, puddings such as semolina, tapioca etc., pasteurised liquid egg, pasteurised potato products, soy products, crumpets, pikelets, flapjacks, processed meat products, beverages, soups, sauces, ready to eat meals, canned foods, vegetable drinks) and low acid foods such as salad dressings, sauces, mayonnaise, beer, wine and other beverages.

Although some loss of activity may be expected when used with processed foods, this may be ameliorated e.g. by increasing the amount of nisin applied. Effective levels of nisin to preserve foodstuffs reportedly range from 25-500 IU/g or more. Other effective levels would be appreciated by one skilled in the art. For example levels of 50-400 IU/g may be utilised.

Since the discovery of the first bacteriocin, nisin, many other bacteriocins have now been found (Hoover, 1993; Ray & Daeschel, 1994; Axelsen, 1998; Naidu, 2000; Ray *et al.* 2001; Ray & Miller, 2003). The bacteriocin pediocin, produced by *Pediococcus pentosaceus*, *P. acidilactici*, or *Lactobacillus plantarum*, may be used in the present invention. Like nisin, different structures of pediocin have been described. At present pediocin and other bacteriocins are not allowed as food additives but their antibacterial

activity can be achieved by production of the bacteriocin *in situ*, as a consequence of the growth of the producer organism in the food. This is the purpose of commercial protective cultures such as HOLDBAC™ Listeria (Danisco). Pediocin has a more narrow antimicrobial spectrum compared to nisin, but there is much interest in its food safety ability to kill, prevent or control the growth of the food pathogen *Listeria monocytogenes* (Ray & Miller, 2000). Other bacteriocins may be used in the present invention, including those named generally as divercin, leucocin, mesentericin, sakacin, curvacin, bavaricin, acidocin, bifidocin, carnobacteriocin, pisicocin, piscicolin, mundticin, enterocin, thermophilin, lacticin, plantaricin, lactococcin, dricin, diplococcin, mesenterocin, leuconosin, carnosin, acidophilin, lactacin, brevicin, lactocin, helevticin, reutericin, propionicin.

MICROORGANISM

As discussed herein the present invention may prevent and/or inhibit the growth of, and/or kill a micro-organism in a material. This may be slowing or arresting a micro-organism, such a bacteria, or by killing the micro-organism present on contact with the present composition.

In one aspect the antimicrobial material is present in an amount to provide a microbicidal or microbiostatic effect.

In one aspect the bacteriocin and the extract are present in an amount to provide a microbicidal or microbiostatic effect.

In a highly preferred aspect the microbicidal or microbiostatic effect is a bactericidal or bacteriostatic effect.

It is advantageous for the bactericidal or bacteriostatic effect to be in respect of Gram-positive bacteria and Gram-negative bacteria. Preferably the bactericidal or bacteriostatic effect is in respect of Gram-positive bacteria.

In a preferred aspect the bactericidal or bacteriostatic effect is in respect of an organism selected from Gram-positive bacteria associated with food spoilage or foodborne disease including *Bacillus* species, *Bacillus subtilis*, *Bacillus cereus*, *Listeria* species, *Listeria*

monocytogenes, lactic acid bacteria, lactic acid spoilage bacteria, *Lactobacillus species*, *Staphylococcus aureus*, *Clostridium* species, *C. sporogenes*, *C. tyrobutyricum* and *C. botulinum* (when the antimicrobial material is recognised as effective against *C. botulinum* or is part of a system effective against *C. botulinum*).

In a preferred aspect the bactericidal or bacteriostatic effect of the invention in combination with a chelating agent is in respect of an organism selected from other micro-organisms associated with food spoilage or foodborne disease, including yeasts, moulds and Gram-negative bacteria including *Escherichia coli*, *Salmonella* species, and *Pseudomonas* species.

In a preferred aspect the bactericidal or bacteriostatic effect is in respect of lactic acid bacteria such as *Lactobacillus*, *Leuconostoc*, *Carnobacterium*, and *Enterococcus*; *Listeria monocytogenes*, spore forming heat resistant bacteria such as *Bacillus* and *Clostridium*; and *Brochothrix thermosphacta*.

In a preferred aspect the bactericidal or bacteriostatic effect is in respect of *Lactobacillus*, *Leuconostoc*, *Carnobacterium*, *Enterococcus*, *Listeria monocytogenes*, *Bacillus*, *Clostridium*; and *Brochothrix thermosphacta*.

In a preferred aspect the bactericidal or bacteriostatic effect is in respect of *Listeria monocytogenes*.

ENCAPSULATED ANTIMICROBIAL MATERIAL

In a preferred aspect the encapsulated antimicrobial material is a particulate form.

Particle size may be important either in the injection aspect of the present invention or the tumbling aspect. The choice of particle size, for example to a particular maximum average particle size, may assist in the introduction of the encapsulated antimicrobial material into the foodstuff. We have found that in the injection aspect of the particle size is particularly important. The particle size, and in particular the maximum average particle size, may determine the likelihood that the shell of the encapsulated antimicrobial material will withstand an injection process.

In a preferred aspect the encapsulated antimicrobial material has an average particle size of less than 500µm, preferably less than 300µm, preferably less than 250µm, preferably less than 150µm, preferably from 50 to 150µm. In some aspects the encapsulated antimicrobial material has an average particle size of less than 100µm, or less than 50µm, or less than 25µm.

As discussed above an aim of the present invention is to provide for the introduction of the antimicrobial material into the foodstuff in a form protected from degradation or inactivation. However, the antimicrobial material should of course be released when required so as to provide the antimicrobial effect which is its purpose. Thus in one preferred aspect the shell is selected to provide sustained release of the antimicrobial material from the encapsulated antimicrobial material.

In one aspect of the present invention the shell is selected to provide sustained release of the antimicrobial material from the encapsulated antimicrobial material or to provide release under predetermined conditions. Suitable predetermined temperature conditions are: greater than 50°C, preferably greater than 60°C, preferably greater than 70°C, preferably greater than 72°C, preferably greater than 75°C, preferably from 72 to 78°C

In one aspect of the present invention the shell is selected to prevent, reduce or inhibit degeneration or inactivation of the antimicrobial material. Preferably the degeneration which is to be prevented is by one or more factors selected from heat degradation, pH induced degradation, protease degradation and glutathione adduct formation.

The shell is or comprises or may be formed from any suitable material. Preferred materials are selected from fats, emulsifiers, waxes (animal, vegetable, mineral or synthetic), liposome-forming lipids (such as glycerophospholipids and sterols), hydrocolloids, natural or synthetic polymers and mixtures thereof. Preferred materials are materials that are brine-insoluble or can be rendered brine insoluble by crosslinking, sintering or other means.

Preferably the glycerophospholipids are selected from phosphatidycholines, phosphatidyethanolamines and phosphatidylglycerols.

Preferably the sterols are selected from cholesterol, ergosterol, lanosterol, and

stigmasterol.

Preferably the fat is a triglyceride, more preferably a vegetable triglyceride.

Preferably the emulsifier is selected from polysorbates, monoglycerides, diglycerides, acetic acid esters of mono-diglycerides, tartaric acid esters of mono-diglycerides and citric acid esters of mono-diglycerides.

Preferably the hydrocolloid is cross linked.

The cross-linking of the hydrocolloids may be is carried out by using cross-linking agents or by a variety of mechanisms. If the hydrocolloid is a protein or polysaccharide bearing amino groups, it can be cross-linked by using dialdehydes, such as glutaraldehyde. If the hydrocolloid is a polysaccharide, such as sodium alginate, gellan gum or pectin, it can be cross-linked with multivalent ions, such as calcium or magnesium. The cross-linking can also be carried out by other mechanisms, such as heating, pH adjustment, applying pressure or by enzymatic cross-linking. Proteins, for example, can be cross-linked by subjecting a protein to a high pressure, preferably from 5 to 200 bar, and/or by subjecting a protein to a temperature which is above the denaturation temperature of the protein. The enzymatic cross-linking of proteins can be carried out for example with transglutamidase. Based on the hydrocolloid used, a person skilled in the art is able to decide which method of gelling or cross-linking is used.

Preferably the hydrocolloid is selected from carrageenan

In one aspect the hydrocolloid is selected from alginate, carrageenan, carboxymethyl cellulose (CMC), guar gum, locust bean gum (LBG), xanthan gum, microcrystalline cellulose (MCC), methyl cellulose (MC), cellulose ethers including hydroxy propyl methyl cellulose (HPMC), pectin, starch including native and modified starch, pregelatinated starch and non-pregelatinated starch, including starch from corn, potato, tapioca, wheat, and rice, gelatin, agar, and combinations thereof.

In one aspect the hydrocolloid is brine-insoluble, particularly at the temperature of use, or a hydrocolloids rendered insoluble by crosslinking.

Preferably the natural or synthetic polymer is selected from shellac, polyvinyl acetate, polymethyl-metacrylate and its derivatives, any brine-insoluble polymers.

In one further preferred aspect the shell is or comprises or may be formed from the group comprising fats, oils, waxes, resins, emulsifiers or mixtures thereof, which are preferably food-grade. Preferably the hydrophobic shell matrix is selected from the group comprising animal oils and fats, fully hydrogenated vegetable or animal oils, partially hydrogenated vegetable or animal oils, unsaturated, hydrogenated or fully hydrogenated fatty acids, unsaturated, partially hydrogenated or fully hydrogenated fatty acid monoglycerides and diglycerides, unsaturated, partially hydrogenated or fully hydrogenated esterified fatty acids of monoglycerides or diglycerides, unsaturated, partially hydrogenated or fully hydrogenated free fatty acids, other emulsifiers, animal waxes, vegetable waxes, mineral waxes, synthetic waxes, natural and synthetic resins and mixtures thereof.

Animal oils and fats are such as, but not restricted to, beef tallow, mutton tallow, lamb tallow, lard or pork fat, sperm oil. Hydrogenated or partially hydrogenated vegetable oils are such as, but not restricted to, canola oil, cottonseed oil, peanut oil, corn oil, olive oil, soybean oil, sunflower oil, safflower oil, coconut oil, palm oil, linseed oil, tung oil and castor oil. Free fatty acids are such as, but not restricted to, stearic acid, palmitic acid and oleic acid. Other emulsifiers are such as, but not restricted to, polyglycerol esters, sorbitan esters of fatty acids. Animal waxes are such as, but not restricted to, beeswax, lanolin, shell wax or Chinese insect wax. Vegetable waxes are such as, but not restricted to, carnauba, candelilla, bayberry or sugarcane waxes. Mineral waxes are such as, but not restricted to, paraffin, microcrysalline petroleum, ozocerite, ceresin or montan. Synthetic waxes are such as, but not restricted to, low molecular weight polyolefin, polyol ether-esters and Fisher-Tropsch process synthetic waxes. Natural resins are such as rosin, balsam, shellac and zein.

In one further preferred aspect the shell is or comprises or may be formed from the group comprising hydrocolloids, sodium alginate, gum arabic, gellan gum, starch, modified starch, guar gum, agar gum, pectin, amidified pectin, carrageenan, xanthan, gelatine, chitosan, mesquite gum, hyaluronic acid, cellulose derivatives such as cellulose acetate phtalate, hydroxy propyl methylcellulose (HPMC), methyl cellulose, ethyl cellulose and carboxy methyl cellulose (CMC), methyl acrylic copolymers, such as Eudragit®, psyllium,

tamarind, xanthan, locust bean gum, whey protein, soy protein, sodium caseinate, any food-grade protein, shellac, zein, any synthetic or natural water-soluble polymers, any water-insoluble microparticles, such as silicone dioxide, titanium dioxide, synthetic or natural food-grade polymer beads and mixtures thereof.

The encapsulated antimicrobial material may be prepared by any suitable process. In one preferred aspect the encapsulated antimicrobial material is prepared by or is obtainable by a process selected from spray cooling, fluidised bed coating, and simple or complex co-acervation.

The coacervation of the encapsulating material, such as hydrocolloid, is carried out by using any suitable coacervation process. The coacervation can be performed for example by adding salt(s), sugar(s), or other additives, which cause the phase separation of the encapsulation material, such as the hydrocolloid(s). The coacervation can also be performed by subjecting the emulsion to heating, cooling, pH change by adding acid or base, which cause the phase separation of the encapsulating material(s), such as the hydrocolloid(s). The deposition of the coacervated phase. The coacervate layer can afterwards be subjected to cross-linking or hardening by any suitable means, which are known to persons skilled in coacervation.

The encapsulating materials suitable for coacervation may be selected form the group comprising shellac, zein, any synthetic or natural hydrophobic polymers, fats, emulsifiers, waxes, any mixture of oppositely charged hydrocolloids, such as gelatine/arabic gum, gelatine/CMC, any proteins/ionic hydrocolloids, any combination of hydrocolloids and a solubility-reducing agent such as salts, sugars, acids or bases, or mixtures thereof.

Further preferred aspects include

(a) spray cooling in fats, waxes or emulsifiers

(b) fluidised bed coating with acid-stable shellac coating, fats, waxes, or emulsifiers, or any other hydrophobic and/or acid stable coating

(c) complex or simple co-acervation in cross-linked hydrocolloids.

In one preferred aspect the shell of the encapsulated antimicrobial material is capable of withstanding injection into the foodstuff.

In one preferred aspect the shell of the encapsulated antimicrobial material is capable of withstanding a pressure of greater than 1.5 bar, for example. greater than 2.0 bar, for example greater than 3.0 bar.

In one preferred aspect the shell of the encapsulated antimicrobial material is capable of withstanding a shear force of greater than that typically encountered during injection.

As discussed herein the shell of the encapsulated antimicrobial material may be selected to provide sustained release of the antimicrobial material from the encapsulated antimicrobial material or to provide release under predetermined conditions. Furthermore the shell of the encapsulated antimicrobial material may be selected to prevent, reduce or inhibit degeneration or inactivation of the antimicrobial material. Preferably the degeneration which is to be prevented is by one or more factors selected from heat degradation, pH induced degradation, protease degradation and glutathione adduct formation.

We have found that provision of an encapsulated antimicrobial material in which the shell is selected to provide sustained release of the antimicrobial material from the encapsulated antimicrobial material or to provide release under predetermined conditions is advantageous irrespective of the manner in which the encapsulated antimicrobial material is contacted with a foodstuff. For example the encapsulated antimicrobial material may be contacted with a foodstuff (or other material) by means other than injection or tumbling. In other words, we have provided an encapsulated antimicrobial material in which antimicrobial material is released in a sustained manner or when the encapsulated antimicrobial material is placed under predetermined conditions.

Thus in a further aspect the present invention provides an antimicrobial material in an encapsulated form, comprising a core of antimicrobial material and shell of encapsulating material, wherein the shell of encapsulating material is impermeable to the antimicrobial material.

In this and other aspects of the invention, by the term "encapsulated" it is meant the packaging of solid particles or liquid droplets of active ingredient (or particles or droplets containing the active ingredient) within a secondary material so as to fully surround the solid particles or liquid droplets with a protective or functional shell material. This

contrasts with the loose use of the term encapsulated to refer to simple coating. For example Cahill et al. teaches the coating of nisin with a porous matrix of alginate. External material can freely diffuse in the alginate matrix and the coated nisin can easily diffuse out through the large pores of the matrix. This is not "encapsulation" in the present sense.

Each of the preferred aspects described herein are applicable to this aspect of the invention. Particularly preferred aspects include

- the antimicrobial material is an antibacterial material.
- the antimicrobial material is a bacteriocin.
- the antimicrobial material is at least nisin.
- the shell is selected to provide sustained release of the antimicrobial material from the encapsulated antimicrobial material.
- the shell is selected to prevent, reduce or inhibit degeneration or inactivation of the antimicrobial material.
- the shell is selected to release the antimicrobial material from the encapsulated antimicrobial material on contact with a foodstuff, preferably the foodstuff is a marinade.

FOODSTUFF

Many foodstuffs may be protected by the present invention. Typical foodstuffs are raw meat, cooked meat, raw poultry products, cooked poultry products, raw seafood products, cooked seafood products, ready to eat meals, pasta sauces, pasteurised soups, mayonnaise, salad dressings, marinades, oil-in-water emulsions, margarines, low fat spreads, water-in-oil emulsions, dairy products, cheese spreads, processed cheese, dairy desserts, flavoured milks, cream, fermented milk products, cheese, butter, condensed milk products, ice cream mixes, soya products, pasteurised liquid egg, bakery products, confectionery products, fruit products, and foods with fat-based or water-containing fillings.

In one preferred aspect the foodstuff is selected from raw meat, cooked meat, raw poultry products, cooked poultry products, raw seafood products, cooked seafood products and raw or cooked foodstuffs prone to surface bacterial growth.

In one preferred aspect the foodstuff is raw meat.

In one preferred aspect the foodstuff comprises whole meat muscle.

ADDITIONAL COMPONENTS

Typically the encapsulated antimicrobial material will not be introduced into the foodstuff alone. Thus in one aspect the encapsulated antimicrobial material is introduced into the foodstuff in a carrier. Preferably the carrier is or comprises brine.

The density of the encapsulated antimicrobial material should match the density of the carrier (such as brine) to avoid separation or sedimentation of the encapsulated antimicrobial material, preventing even distribution of encapsulated antimicrobial material during injection or tumbling. Thus in a preferred aspect the carrier and the encapsulated antimicrobial material have substantially the same density.

Matching the density of the carrier and the encapsulated antimicrobial material may be achieved by careful selection of carrier and encapsulated antimicrobial material. Alternatively it may be achieved by modification of the encapsulated antimicrobial material to have substantially the same density as the carrier, or by modification of the carrier to have substantially the same density as the encapsulated antimicrobial material. The encapsulated antimicrobial material may be modified by contacting the encapsulated antimicrobial material with oil, such as a brominated oil. The carrier may be modified by inclusion of an additional component such as xanthum gum.

The carrier may contain one or more additional components. However, in some aspects the carrier contains no additional components or contains no additional components that materially affect the properties of the composition.

In one preferred aspect the carrier further comprises an emulsifier. Preferably the emulsifier is selected from polyoxy-ethylene sorbitan esters (E432-E436) otherwise known as polysorbates (e.g. Tween 80, Tween 20), monoglycerides, diglycerides, acetic acid esters of mono-diglycerides, tartaric acid esters of mono-diglycerides and citric acid esters of mono-diglycerides.

The encapsulated antimicrobial material may contain one or more additional components. However, in some aspects the encapsulated antimicrobial material contains no additional components or contains no additional components that materially affect the properties of the composition.

In one preferred aspect the encapsulated antimicrobial material further comprises a chelator. Preferably the chelator is selected from EDTA, citric acid, monophosphates, diphosphates, triphosphates and polyphosphates.

Further suitable chelator are taught in US 5573801 and include carboxylic acids, polycarboxylic acids, amino acids and phosphates. In particular, the following compounds and their salts may be useful:

Acetic acid, Adenine, Adipic acid, ADP, Alanine, B-Alanine, Albumin, Arginine, Ascorbic acid, Asparagine, Aspartic acid, ATP, Benzoic acid, n-Butyric acid, Casein, Citraconic acid, Citric acid, Cysteine, Dehydracetic acid, Desferri-ferrichrysin, Desferri-ferrichrome, Desferri-ferrioxamin E, 3,4-Dihydroxybenzoic acid, Diethylenetriaminepentaacetic acid (DTPA), Dimethylglyoxime, O,O-Dimethylpurpurogallin, EDTA, Formic acid, Fumaric acid, Globulin, Gluconic acid, Glutamic acid, Glutaric acid, Glycine, Glycolic acid, Glycylglycine, Glycylsarcosine, Guanosine, Histamine, Histidine, 3-Hydroxyflavone, Inosine, Inosine triphosphate, Iron-free ferrichrome, Isovaleric acid, Itaconic acid, Kojic acid, Lactic acid, Leucine, Lysine, Maleic acid, Malic acid, Methionine, Methylsalicylate, Nitrilotriacetic acid (NTA), Ornithine, Orthophosphate, Oxalic acid, Oxystearin, B-Phenylalanine, Phosphoric acid, Phytate, Pimelic acid, Pivalic acid, Polyphosphate, Proline, Propionic acid, Purine, Pyrophosphate, Pyruvic acid, Riboflavin, Salicylaldehyde, Salicyclic acid, Sarcosine, Serine, Sorbitol, Succinic acid, Tartaric acid, Tetrametaphosphate, Thiosulfate, Threonine, Trimetaphosphate, Triphosphate, Tryptophan, Uridine diphosphate, Uridine triphosphate, n-Valeric acid, Valine, and Xanthosine

Many of the above sequestering agents are useful in food processing in their salt forms, which are commonly alkali metal or alkaline earth salts such as sodium, potassium or calcium or quaternary ammonium salts. Sequestering compounds with multiple valencies may be beneficially utilised to adjust pH or selectively introduce or abstract metal ions e.g. in a food system coating. Additional information chelators is disclosed in

T. E. Furia (Ed.), CRC Handbook of Food Additives, 2nd Ed., pp. 271-294 (1972, Chemical Rubber Co.), and M. S. Peterson and A. M. Johnson (Eds.), Encyclopaedia of Food Science, pp. 694-699 (1978, AVI Publishing Company, Inc.) which articles are both hereby incorporated by reference.

The terms "chelator" is defined as organic or inorganic compounds capable of forming co-ordination complexes with metals. Also, as the term " chelator" is used herein, it includes molecular encapsulating compounds such as cyclodextrin. The chelator may be inorganic or organic, but preferably is organic.

Preferred chelator are non-toxic to mammals and include aminopolycarboxylic acids and their salts such as ethylenediaminetetraacetic acid (EDTA) or its salts (particularly its di- and tri-sodium salts), and hydrocarboxylic acids and their salts such as citric acid. However, non-citric acid and non-citrate hydrocarboxylic acid chelators are also believed useful in the present invention such as acetic acid, formic acid, lactic acid, tartaric acid and their salts.

As noted above, the term "chelator" is defined and used herein as a synonym for sequestering agent and is also defined as including molecular encapsulating compounds such as cyclodextrin. Cyclodextrins are cyclic carbohydrate molecules having six, seven, or eight glucose monomers arranged in a donut shaped ring, which are denoted alpha, beta or gamma cyclodextrin, respectively. As used herein, cyclodextrin refers to both unmodified and modified cyclodextrin monomers and polymers. Cyclodextrin molecular encapsulators are commercially available from American Maize-Products of Hammond, Ind. Cyclodextrin are further described in Chapter 11 entitled, "Industrial Applications of Cyclodextrin", by J. Szejtli, page 331-390 of Inclusion Compounds, Vol. III (Academic Press, 1984) which chapter is hereby incorporated by reference.

Preferably the chelator enhances the antimicrobial activity and/or antimicrobial spectrum of the bacteriocin. More preferably the chelator enhances the antimicrobial activity and/or antimicrobial spectrum of the bacteriocin in respect of Gram-negative bacteria and other micro-organisms.

We have found that the provision of a chelator is particularly effective in view of the enhancement of the antimicrobial activity and/or antimicrobial spectrum of the bacteriocin

provided. This enhancement is possible irrespective of the manner in which the encapsulated antimicrobial material is delivered or the nature of the shell of the encapsulated antimicrobial material

Thus in a further aspect the present invention provides an antimicrobial material in an encapsulated form, comprising (a) a core of (i) an antimicrobial material and (ii) a chelator; and (b) a shell of encapsulating material.

Each of the preferred aspects described herein are applicable to this aspect of the invention. Particularly preferred aspects include

- wherein the shell of encapsulating material is impermeable to the antimicrobial material.
- the shell is selected to provide sustained release of the antimicrobial material from the encapsulated antimicrobial material.
- the shell is selected to prevent, reduce or inhibit degeneration or inactivation of the antimicrobial material.
- the chelator enhances the antimicrobial activity and/or antimicrobial spectrum of the bacteriocin
- the chelator enhances the antimicrobial activity and/or antimicrobial spectrum of the bacteriocin in respect of Gram-negative bacteria and other micro-organisms.
- the chelator is selected from EDTA, citric acid, monophosphates, diphosphates, triphosphates and polyphosphates.
- the antimicrobial material is an antibacterial material.
- the antimicrobial material is a bacteriocin.
- the antimicrobial material is at least nisin.

PROCESS

The encapsulated antimicrobial material may be introduced into the foodstuff by (a) injecting the encapsulated antimicrobial material into the foodstuff or (b) tumbling the encapsulated antimicrobial material with the foodstuff.

In one aspect the encapsulated antimicrobial material is introduced into the foodstuff by injecting the encapsulated antimicrobial material into the foodstuff.

In one aspect the encapsulated antimicrobial material is introduced into the foodstuff by tumbling the encapsulated antimicrobial material with the foodstuff.

As noted herein the encapsulated antimicrobial material may be introduced into the foodstuff by means other than injection or tumbling. For example the encapsulated antimicrobial material may be incorporated in a marinade. Marinated meat can be prepared in two ways: 1) a surface treatment (such as, for example but not limited to, adding the marinade to the raw meat followed by gas- or vacuum packing) or 2) forceful incorporation on the marinade/brine by physical means (such as, for example but not limited to, tumbling or injection).

Teachings on the practice of injection into foodstuffs or tumbling of foodstuffs can be found in WO 00/62632.

HIGHLY PREFERRED ASPECTS

Some highly preferred aspects of the present invention are set out below

- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff.
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus*, *Leuconostoc*, *Carnobacterium*, *Enterococcus*; *Listeria monocytogenes*, *Bacillus*, *Clostridium*; and *Brochothrix thermosphacta*.
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the

encapsulated nisin with the foodstuff, wherein the encapsulated nisin has an average particle size of less than 150µm.

- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the shell is or comprises a material selected from triglyceride and carrageenan
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the foodstuff is raw meat.
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the encapsulated nisin is introduced into the foodstuff in a brine carrier
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Carnobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the encapsulated nisin has an average particle size of less than 150µm.
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Carnobacterium, Enterococcus; Listeria*

monocytogenes, Bacillus, Clostridium; and *Brochothrix thermosphacta*, wherein the shell is or comprises a material selected from triglyceride and carrageenan

- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Camobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the foodstuff is raw meat.
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Carnobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the encapsulated nisin is introduced into the foodstuff in a brine carrier
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the encapsulated nisin has an average particle size of less than 150μm, wherein the shell is or comprises a material selected from triglyceride and carrageenan
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the encapsulated nisin has an average particle size of less than 150μm, wherein the foodstuff is raw meat.
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of

encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the encapsulated nisin has an average particle size of less than 150µm, wherein the encapsulated nisin is introduced into the foodstuff in a brine carrier

- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the shell is or comprises a material selected from triglyceride and carrageenan, wherein the foodstuff is raw meat.
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the shell is or comprises a material selected from triglyceride and carrageenan, wherein the encapsulated nisin is introduced into the foodstuff in a brine carrier
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the foodstuff is raw meat, wherein the encapsulated nisin is introduced into the foodstuff in a brine carrier
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Carnobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the foodstuff is raw meat, wherein the encapsulated nisin has an average particle size of less than 150µm.
- a process for introducing an antimicrobial material into a foodstuff comprising (i)

providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Camobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the foodstuff is raw meat, wherein the shell is or comprises a material selected from triglyceride and carrageenan

- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Camobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the foodstuff is raw meat, wherein the encapsulated nisin is introduced into the foodstuff in a brine carrier
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Camobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the encapsulated nisin is introduced into the foodstuff in a brine carrier, wherein the encapsulated nisin has an average particle size of less than 150µm.
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism

selected from *Lactobacillus, Leuconostoc, Carnobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the encapsulated nisin is introduced into the foodstuff in a brine carrier, wherein the shell is or comprises a material selected from triglyceride and carrageenan

- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Carnobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the encapsulated nisin is introduced into the foodstuff in a brine carrier, wherein the foodstuff is raw meat.
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Carnobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the encapsulated nisin has an average particle size of less than 150μm, wherein the shell is or comprises a material selected from triglyceride and carrageenan, wherein the foodstuff is raw meat, wherein the encapsulated nisin is introduced into the foodstuff in a brine carrier
- an antimicrobial material in an encapsulated form, comprising a core of nisin and shell of encapsulating material, wherein the shell of encapsulating material is impermeable to nisin
- an antimicrobial material in an encapsulated form, comprising a core of nisin and shell of encapsulating material, wherein the shell of encapsulating material is impermeable to nisin, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Carnobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*.

- an antimicrobial material in an encapsulated form, comprising a core of nisin and shell of encapsulating material, wherein the shell of encapsulating material is impermeable to nisin, wherein the encapsulated nisin has an average particle size of less than 150μm.
- an antimicrobial material in an encapsulated form, comprising a core of nisin and shell of encapsulating material, wherein the shell of encapsulating material is impermeable to nisin, wherein the shell is or comprises a material selected from triglyceride and carrageenan
- an antimicrobial material in an encapsulated form, comprising a core of nisin and shell of encapsulating material, wherein the shell of encapsulating material is impermeable to nisin, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Carnobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the encapsulated nisin has an average particle size of less than 150μm.
- an antimicrobial material in an encapsulated form, comprising a core of nisin and shell of encapsulating material, wherein the shell of encapsulating material is impermeable to nisin, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Carnobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the shell is or comprises a material selected from triglyceride and carrageenan
- an antimicrobial material in an encapsulated form, comprising a core of nisin and shell of encapsulating material, wherein the shell of encapsulating material is impermeable to nisin, wherein the encapsulated nisin has an average particle size of less than 150μm, wherein the shell is or comprises a material selected from triglyceride and carrageenan
- an antimicrobial material in an encapsulated form, comprising a core of nisin and shell of encapsulating material, wherein the shell of encapsulating material is impermeable to nisin, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Carnobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the foodstuff is raw meat, wherein the encapsulated nisin has an average particle size of less than 150μm.

- an antimicrobial material in an encapsulated form, comprising a core of nisin and shell of encapsulating material, wherein the shell of encapsulating material is impermeable to nisin, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Carnobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the foodstuff is raw meat, wherein the shell is or comprises a material selected from triglyceride and carrageenan

The present invention will now be described in further detail in the following examples.

EXAMPLES

Example 1

First, a solution of 15 g k-carrageenan in 1000 mL of phosphate buffer at pH 3,5 is prepared at 85°C. To this is added 300 g of Nisaplin® (Danisco commercial extract of nisin: equivalent to 1 x 10^6 IU/g nisin potency). The resulting mixture is thoroughly mixed. At the same time, a mixture of 1333 g of a vegetable triglyceride (Danisco: GRINSTED ® PS 101, m.p. 58°C) and 73 g of acetylated emulsifier (Danisco: Acetem 50 00) is melted at 85°C in a water bath. The melted fat mixture is kept under homogenisation (Silverson mixer, 8 kRPM) as the aqueous mixture is slowly incorporated. The homogenisation is maintained for 5 minutes after the whole aqueous mixture is added and then a solution of 3 g of polysorbate 80 in 40 mL of water is added under constant mixing. The resulting low-viscosity water-in-oil emulsion is then immediately spray cooled in a Niro spray tower using the following parameters: inlet air temperature: 10°C, outlet air temperature 28 °C, rotating atomization wheel speed: 10 kPRM. A free flowing powder is obtained.

This encapsulated nisin can be used for injection or tumbling of raw meat that is then immediately cooked. Nisin release from the fat shell would occur upon injection and/or cooking. Since the fat-based encapsulated shell material would make the particles float to the surface of the injection brine, either a) a viscosifying agent such as xanthan could be used to stabilise the particles in the brine, or b) to mix the brine before use as an

injection material. Mixing of the particles would naturally occur when encapsulated nisin is used in the brine used for tumbling of meat.

The same encapsulated material can be used for sustained release at chilled temperature of encapsulated nisin within marinades used on vacuum-packaged, chilled raw meat.

Example 2

First, a solution of 15 g sodium alginate in 1000 mL of phosphate buffer at pH 3.5 is prepared at 85°C. To this is added 300 g of Nisaplin® (Danisco commercial extract of nisin: equivalent to 1 x 10^6 IU/g nisin potency). The resulting mixture is thoroughly mixed. At the same time, a mixture of 1333 g of a vegetable triglyceride (Danisco: GRINSTED ® PS 101, melting point 58 °C) and 73 g of acetylated emulsifier (Danisco: Acetem 50 00) is melted at 85 °C in a water bath. The melted fat mixture is kept under homogenization (Silverson mixer, 8 kRPM) as the aqueous mixture is slowly incorporated. Following the incorporation of the aqueous mixture, a solution of 7 g of calcium chloride in 70 mL of water is added dropwise. The homogenization is maintained for another 5 minutes and then a solution of 3 g of polysorbate 80 in 40 mL of water is added under constant mixing. The resulting low-viscosity water-in-oil emulsion is then immediately spray cooled in a Niro spray tower using the following parameters: inlet air temperature: 10°C, outlet air temperature 28°C, rotating atomization wheel speed: 10 kPRM. A free flowing powder is obtained.

The use of this encapsulated nisin is as described in Example 1.

Example 3

A solution of 1 g of a bilayer-forming lipid and 100 mg of cholesterol in a suitable organic solvent is evaporated so as to form a thin dry lipid film on the bottom of the container. After thorough drying of the lipid film, 1 L of water containing nisin (as Nisaplin®) at the saturation concentration is added to the container and the mixture is thoroughly mixed or homogenized. The resulting suspension of multilamellar vesicle (MLV) can be further processed by microfluidization to form smaller more homogenous small unilamellar

vesicle (SUV). The suspension of liposome-encapsulated nisin can be added directly to the meat by injection/tumbling.

These particles are small enough to pass through injection needles without disintegration of the liposome shell. The liposome-encapsulated nisin would be released on cooking since liposomes break up at 45–50°C because of the transition temperature of bilayer-forming phospholipids/amphiphilic compounds. Lipsome-encapsulated nisin would be slowly released over time, thus making it suitable for sustained release in raw meat marinades.

Liposome-encapsulated nisin can be made by several processes, including microfluidization, extrusion, 'French press', reverse phase evaporation, freeze-thaw cycle, etc. Microfluidization, is the preferred aspect since it is a continuous, high capacity and solvent –free process.

Example 4

Use of a fluidised bed to apply a hydrophobic shell onto the nisin. If the nisin particle size is too fine, the powder can be agglomerated in an suitable equipment using a binder solution (solution of sticky hydrocolloids such as alginate or maltodextrin) in order to obtain a dense powder of particle size between 100-150 micrometres. The appropriate powder is then introduced into the coating chamber of a fluidized-bed microencapsulation unit and fluidized at inlet air flow rate of 5-30 cm/s and temperature up to 50°C to fluidized the particles. A coating material is then sprayed onto the fluidized bed of antimicrobial using a double fluid nozzle and high pressure atomization air.

In one example, a melted mixture of triglyceride and additives is sprayed onto the nisin to form a continuous layer of fat around each individual particle as the melted fat spread and solidifies on the particles. The amount of fat applied can be up to 50%, but no usually no lower than 20% w/w.

In another example, a dispersion of coating material in water or a solution of coating material in ethanol is sprayed onto the fluidized particles and the fluidization air is used to evaporate the solvent or the water, which leaves behind a continuous film of coating

material on the antimicrobial particles. Examples of coating material in this case include shellac, zein or any other hydrophobic coating materials.

In order for encapsulated nisin prepared by this method to used for raw meat injection, the particles size must be less than 175 micrometres. In addition, the particle size must be greater than 100 micrometres for the fluidization process to work.

REFERENCES

Background

- Davies, E. A., Bevis, H. E., Potter, R., Harris, J., Williams, G. C. and Delves-Broughton, J. 1998. The effect of pH on the stability of nisin solutions during autoclaving. Letters in Applied Microbiology 27: 186-187.
- De Vuyst, L., and Vandamme, E. J. 1994. Nisin, a lantibiotic produced by Lactococcus lactis subsp lactis: properties, biosynthesis, fermentation and applications. In Bacteriocins of Lactic Acid Bacteria. Microbiology, Genetics and Applications, eds. L. de Vuyst and E. J. Vandamme pp 151 – 221. London: Blackie Academic and Professional.
- Rose, N. L., Palcic, M. M., Sporns, P. and McMullen. 2002. Nisin: a novel substrate for glutathione S-transferase isolated from fresh beef. Journal of Food Safety 67:2288-2293.
- Rose, N. L., Sporns, P., Stiles, M. E., and McMullen, L. 1999. Inactivation of nisin by glutathione in fresh meat. J. Food Science 64: 759-762.
- Rose, N. L., Sporns, P., Dodd, H. M., Gasson, M. J., Mellon, F. A., and McMullen, L. 2003. Involvement of dehydroalanine and dehydrobutyrine in the addition of glutathione to nisin. J. Agric Food chem. 51: 3174 – 3178.
- Susiluoto, T., Korkeala, H., and Bjorkroth, K. J. 2003. Leuconstoc gasicomitatum is the dominating lactic acid bacterium in retail modified atmosphere packaged marinated broiler meat strips on sell by day. International Journal of Food Microbiology 80: 89-97.
- Thomas, L. V., Clarkson, M. R., and Delves-Broughton, J. 2000. Nisin. In: Natural Food Antimicrobial Systems. Ed. A. S. Naidu. Pp 463-524. USA: CRC Press.
- Varnam, A. H., and Sutherland, J. P. 1995. Meat and Meat Products. Technology, Chemistry and Microbiology. Chapman & Hall. London.
- Axelsen, L. 1998. Lactic acid bacteria: classification and physiology'. In: Salminen, S. and von Wright, A. In: Lactic Acid Bacteria. 2nd Ed. New York, Marcel Dekker, pp 1-72.
- Delves-Broughton, J. 1990. Nisin and its uses as a food preservative. Food Technol. 44: 100, 102, 104, 106, 108, 111-112, 117.
- Hoover, D. G. 1993. Bacteriocins with potential for use in foods. In: Antimicrobials in Foods. Ed: P. M. Davidson and A. L. Branen. Marcel Dekker, USA.
- Hurst, A. 1981. Nisin. Adv. Appl. Microbiol. 27: 85-123

- Hurst, A. 1983. Nisin and other inhibitory substances from lactic acid bacteria. In Antimicrobials in Foods. Eds. A. L. Branen and P. M. Davidson, pp. 327-351. New York: Marcel Dekker.
- Naidu, A. S. (Ed.) 2000. Natural Food Antimicrobial Systems. USA: CRC Press.
- Ray, B., and Miller, K. W. 2003. Bacteriocins other than nisin: the pediocin-like cystibiotics of lactic acid bacteria. In: Natural Antimicrobials for the Minimal Processing of Foods. Ed: Sibel Roller. CRC Press, USA.
- Ray, B. and Daeschel, M. A. 1994. Bacteriocins of starter culture bacteria. In: Natural Antimicrobial Ssytems and Food Preservation. 1994. Ed: Dillon, V. M. and Board, R. G. CAB International, UK, pp 133 – 166.
- Ray, B., Miller, K. W. and Jain, M. K. 2001. Bacteriocins of lactic acid bacteria. Indian Journal of Microbiology 41: 1-21.
- Thomas, L. V., and Delves-Broughton, J. 2001. New advances in the application of the food preservative nisin. Research Advances in Food Science 2:11-22
- Wessels, S., Jelle, B., and Nes, I. F. 1998. Bacteriocins of the Lactic Acid Bacteria: An Overlooked Benefit for Food. Danish Toxicology Centre, Denmark.

Nisin injection into meat

- Caserio, G., Ciampella, A., Gennari, M., and Barluzzi, A. M. 1979. Industrie Alimentari 18: 1-12. Research on the use of nisin in cooked, cured meat products.
- Gola, J. 1962. 'Preservation of canned hams stored at unusual temperatures'. Collected Reports of Research Institute for Meat (Brno) 10: 239-244.
- Taylor and Somers. 1985. Evaluation of the antibotulinal effectiveness of nisin in bacon. Journal of Food Protection 48:949-952.
- Usborne, WR, Collins-Thompson, DL and Wood, DS. 1986. Sensory evaluation of nisin-treated bacon. Can. Inst. Food Sci. Technol. J. 19: 38-40.
- US 2003/0108648 A1 2003 (Rhodia) 'Composition having bacteriostatic and bactericidal activity against bacterial spores and vegetative cells and process for treating foods therewith'. Ming, King and Payne.
- US 6207210 B1. (Rhodia). 'Broad-range antibacterial composition and process of applying to food surfaces. Bender, King, Ming and Weber. Patent filed March 27, 2001.
- EP 0 770336 A1. European patent application. 1995. Nestle. Process for preparing a meat product.

- Internet article [http://www.nal.usda.gov/fsrio/ppd/ars010f.htm] on work at Meat Research Unit, MARC mentioned a presentation on 'antibacterial properties of injectable beef marinades'. This seems aimed at E. coli O157 and would be unlikely to be nisin.

Encapsulation of nisin

- Benech, R. –O, Kheadr, E. E., Laridi, R., and Fliss, I. 2002. Inhibition of Listeria innocua in cheddar cheese by addition of nisin Z in liposomes or by in situ production in mixed culture. Applied & Environmental Microbiology 68: 3683 – 3690.
- Bower, C. K., McGuire, J. and Daeschel, M. A. 1995. Influences on the antimicrobial activity of surface-adsorbed nisin. J. Industrial Microbiology 15: 227-233.
- Bower, C. K., McGuire, J. and Daeschel, M. A. 1995. Supression of Listeria monocytogenges colonization following adsorption of nisin onto silica surfaces. Appl. Environ. Microbiol 61: 992-997..
- Cahill, S. M., Upton, M. E., and McLoughlin, A. J. 2001. Bioencapsulation technology in meat preservation. In: Applied Microbiology. Eds Durieux, A., and Simon, J. P. Dordrecht: Kluwer Academic Publishers. Pp239-266.
- Cutter, C. N., and Siragusa, G. R. 1996. Reduction of Brochothrix thermosphacta on beef surfaces following immobilization of nisin in calcium gels. Letts. Applied Microbiology 23: 9 – 12.
- Cutter, C. N., and Siragusa, G. R. 1997. Growth of Brochothrix thermosphacta in ground beef following treatments with nisin in calcium alginate gels. Food Microbiol 14: 425-430.
- Cutter, C. N., and Siragusa, G. R. 1998. Incorporation of nisin into meat binding system to inhibit bacteria on beef surfaces. Letts. Applied Microbiol. 27: 19-23.
- Daeschel, M. A., McGuire, J., and Al-Makhlafi, H. 1992. Antimicrobial activity of nisin adsorbed to hydrophilic and hydrophobic silicon surfaces. J. Food Protection 55: 731-735.
- Degnan, A. J., and J. B. Luchansky. 1992. Influence of beef tallow and muscle on the antilisterial activity of pediocin AcH and liposome-encapsulated pediocin AcH. J. Food Protection 55: 552-554.
- Degnan, A. J., Buyong, N., and Luchansky, J. B. 1993. Antilisterial activity of pediocin AcH in model food systems in the presence of an emulsifier or encapsulated within liposomes. International Journal of Food Microbiology 18: 127-138.

- Laridi, R., Benech, R. –O, Vuillemard, J. C., Lacroix, C., Fliss, I. 2003. Liposome encapsulated nisin Z: optimisation, stability and release during milk fermentation. International Dairy Journal. 13: 325-336.
- Lante, A., Crapisi, A., Pasini, G., amd Scalabrini, P. 1994. Nisin released from immobilization matrices as antimicrobial agent. Bitoechnol. Letts 16: 293 – 298.
- Lante, A., Crapisi, A., Zannoni, S., and Spettoli, P. 2000. Nisin released from membrane reactor for dairy Clostridia control. Industrie Alimentari XXXIX: 589-595.
- Robinson, S. K. 1993. Regulatory aspects of bacteriocin use. In Bacteriocins of Lactic Acid Bacteria. Ed. Hoover, DG and Steenson, L. R. pp 233-247. London: Academic Press.
- Shahidi, F. and Han, X. G. 1993. Encapsulation of food ingredients. Critical Review in Food Science and Nutrition 33: 501-547.
- Wan, J., Hickey, M. W. and Coventry, M. J. 1995. Continuous production of bacteriocins, brevicin, nisin and pediocin, using calcium alginate-immobilised bacteria. Journal of Applied Bacteriology 79: 6712-676.
- Wan, J., Gordon, J. B., Muirhead, K., Hickey, M. W., and Coventry, M. J. 1997. Incorporation of nisin in micro-particles of calcium alginate. Letters in Applied Microbiology 24: 153-158.
- WO 02/094224 A1. Bioactive agent + bioactive carbohydrate polymer. Not relevant
- WO 9856402. Ambi. Oral formulation of nisin with a salt in a coating to give release into the colon to treat bacterial infections.
- WO 9720473. Wrigley. Chewing gum with improved flavour using nisin, coating to make a pellet.
- GB2388581A. Microcapsules and method for preparing them. Encapsulation method.

Nisin degradation by proteases

- Alifax, R. and Chevalier, R. 1962. Study of the nisinase produced by Streptococcus thermophilus. J. Dairy Res 29: 233
- Campbell, L. L. 1959. Effect of subtilin and nisin on spores of Bacillus coagulans. J. Bacteriol. 77: 766.
- Jarvis, B. and Mahoney, R. R. 1969. Inactivation of nisin by alpha chymotrypsin. Journal of Dairy Science 52: 1448-1450.
- Jarvis, B. 1967. Resistance to nisin and production of nisin-inactivating enzymes by several Bacillus species. J. Gen Microbiol 47: 33.

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in chemistry, biology, food science or related fields are intended to be within the scope of the following claims

CLAIMS

1. A process for introducing an antimicrobial material into a foodstuff comprising (i) providing the antimicrobial material in an encapsulated form comprising a core of antimicrobial material and shell of encapsulating material (ii) introducing encapsulated antimicrobial material into the foodstuff by (a) injecting the encapsulated antimicrobial material into the foodstuff or (b) tumbling the encapsulated antimicrobial material with the foodstuff.

2. A process according to claim 1 wherein the antimicrobial material is an antibacterial material.

3. A process according to claim 2 wherein the antimicrobial material is a bacteriocin.

4. A process according to claim 3 wherein the bacteriocin is selected from lanthionine containing bacteriocins, *Lactococcus*-derived bacteriocins, *Streptococcus*-derived bacteriocins, *Pediococcus*-derived bacteriocins, *Lactobacillus*-derived bacteriocins, *Carnobacterium*-derived bacteriocins, *Leuconostoc*-derived bacteriocins, *Enterococcus*-derived bacteriocins and mixtures thereof.

5. A process according to any one of the preceding claims wherein the antimicrobial material is at least nisin.

6. A process according to any one of the preceding claims wherein the antimicrobial material is present in an amount to provide a microbicidal or microbiostatic effect.

7. A process according to claim 6 wherein the microbicidal or microbiostatic effect is a bactericidal or bacteriostatic effect.

8. A process according to claim 7 wherein the bactericidal or bacteriostatic effect is in respect of Gram-positive bacteria.

9. A process according to claim 7 wherein the bactericidal or bacteriostatic effect is in respect of an organism selected from species of *Bacillus*, species of *Clostridium*, *Listeria monocytogenes*, lactic acid bacteria, *Leuconostoc*, *Camobacterium*,

Enterococcus; Brochothrix thermosphacta and *Lactobacillus* species.

10. A process according to claim 7 wherein the bactericidal or bacteriostatic effect is in respect of *Listeria monocytogenes.*

11. A process according to any one of the preceding claims wherein the shell of the encapsulated antimicrobial material is capable of withstanding injection.

12. A process according to any one of the preceding claims wherein the shell of the encapsulated antimicrobial material is capable of withstanding a pressure of greater than 1.5 bar.

13. A process according to any one of the preceding claims wherein the encapsulated antimicrobial material is a particulate form.

14. A process according to any one of the preceding claims wherein the encapsulated antimicrobial material has an average particle size of less than 150µm.

15. A process according to any one of the preceding claims wherein the shell is selected to provide sustained release of the antimicrobial material from the encapsulated antimicrobial material.

16. A process according to any one of the preceding claims wherein the shell is selected to prevent, reduce or inhibit degeneration or inactivation of the antimicrobial material.

17. A process according to claim 16 wherein degeneration is by one or more factors selected from heat degradation, pH induced degradation, protease degradation and glutathione adduct formation.

18. A process according to any one of the preceding claims wherein the shell is or comprises a material selected from fats, emulsifiers, waxes (animal, vegetable, mineral or synthetic), liposome-forming lipids, hydrocolloids, natural or synthetic polymers and mixtures thereof.

19. A process according to claim 18 wherein the lipid is a glycerophospholipid or and sterol.

20. A process according to claim 18 or 19 wherein the fat is a triglyceride.

21. A process according to claim 20 wherein the triglyceride is a vegetable triglyceride.

22. A process according to any one of claims 18 to 21 wherein the emulsifier is selected from polysorbates, monoglycerides, diglycerides, acetic acid esters of mono-diglycerides, tartaric acid esters of mono-diglycerides and citric acid esters of mono-diglycerides.

23. A process according to any one of claims 18 to 22 wherein the hydrocolloid is cross linked.

24. A process according to claim 23 wherein the hydrocolloid is carrageenan.

25. A process according to any one of the preceding claims wherein the encapsulated antimicrobial material is prepared by or is obtainable by a process selected from spray cooling, fluidised bed coating, and simple or complex co-acervation.

26. A process according to any one of the preceding claims wherein the foodstuff is selected from raw meat, cooked meat, raw poultry products, cooked poultry products, raw seafood products, and cooked seafood products.

27. A process according to claim 26 wherein the foodstuff is raw meat.

28. A process according to claim 26 or 27 wherein the foodstuff comprises whole meat muscle.

29. A process according to any one of the preceding claims wherein the encapsulated antimicrobial material is introduced into the foodstuff in a carrier.

30. A process according to claim 29 wherein the carrier is or comprises brine.

31. A process according to claim 29 or 30 wherein the carrier and the encapsulated antimicrobial material have substantially the same density.

32. A process according to claim 31 wherein the encapsulated antimicrobial material is modified to have substantially the same density as the carrier.

33. A process according to claim 32 wherein the encapsulated antimicrobial material is modified by contacting the encapsulated antimicrobial material with oil.

34. A process according to claim 33 wherein the oil is brominated oil.

35. A process according to claim 31 wherein the carrier is modified to have substantially the same density as the encapsulated antimicrobial material.

36. A process according to claim 35 wherein the carrier comprises xanthum gum.

37. A process according to any one of claims 31 to 33 wherein the carrier comprises an emulsifier.

38. A process according to any one of the preceding claims wherein the encapsulated antimicrobial material further comprises a chelator.

39. A process according to claim 38 wherein the chelator is selected from EDTA, citric acid, monophosphates, diphosphates, triphosphates and polyphosphates.

40. A process according to claim 38 or 39 wherein the chelator enhances the antimicrobial activity and/or antimicrobial spectrum of the antimicrobial material.

41. A process according to claim 38, 39 or 40 wherein the chelator enhances the antimicrobial activity and/or antimicrobial spectrum of the antimicrobial material in respect of Gram-negative bacteria.

42. A process according to any one of the preceding claims wherein the encapsulated antimicrobial material is introduced into the foodstuff by injecting the

encapsulated antimicrobial material into the foodstuff.

43. A process according to any one of claims 1 to 41 wherein the encapsulated antimicrobial material is introduced into the foodstuff by tumbling the encapsulated antimicrobial material with the foodstuff.

44. An antimicrobial material in an encapsulated form, comprising (i) a core comprising an antimicrobial material and (ii) a shell of encapsulating material, wherein the shell of encapsulating material is impermeable to the antimicrobial material.

45. An antimicrobial material according to claim 44 wherein the antimicrobial material is an antibacterial material.

46. An antimicrobial material according to claim 44 or 45 wherein the antimicrobial material is a bacteriocin.

47. An antimicrobial material according to claim 46 wherein the antimicrobial material is at least nisin.

48. An antimicrobial material according to any one of claims 44 to 47 wherein the shell is selected to provide sustained release of the antimicrobial material from the encapsulated antimicrobial material.

49. An antimicrobial material according to any one of claims 44 to 48 wherein the shell is selected to prevent, reduce or inhibit degeneration or inactivation of the antimicrobial material.

50. An antimicrobial material according to any one of claims 44 to 49 wherein the shell is selected to release the antimicrobial material from the encapsulated antimicrobial material under predetermined conditions.

51. An antimicrobial material according to any one of claims 44 to 50 wherein the shell is selected to release the antimicrobial material from the encapsulated antimicrobial material on contact with a foodstuff.

52. An antimicrobial material according to claim 46 wherein the foodstuff is a marinade.

53. A foodstuff prepared by a process as defined in any one of claims 1 to 43.

54. A foodstuff obtainable by a process as defined in any one of claims 1 to 43.

55. A process as substantially hereinbefore described with reference to any one of the Examples.

56. A foodstuff as substantially hereinbefore described with reference to any one of the Examples.

ABSTRACT

PROCESS

The present invention provides a process for introducing an antimicrobial material into a foodstuff comprising (i) providing the antimicrobial material in an encapsulated form comprising a core of antimicrobial material and shell of encapsulating material, and (ii) introducing encapsulated antimicrobial material into the foodstuff by (a) injecting the encapsulated antimicrobial material into the foodstuff or (b) tumbling the encapsulated antimicrobial material with the foodstuff.